S10.O2

The insertion and assembly of membrane proteins (including subunits of the ATP synthase) by the bacterial holo-translocon

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The SecY/61 complex forms the protein-channel component of the ubiquitous protein secretion and membrane protein insertion apparatus. The bacterial version SecYEG interacts with the highly conserved YidC and SecDF-YajC sub-complex, which facilitate translocation into and across the membrane. Together, they form a super-complex aka the holo-translocon (HTL), which we have successfully over-expressed and purified. Its availability has enabled a comprehensive analysis of its structure and activity, much of which is unpublished. The HTL complex supports ATP and proton-motive-force driven protein secretion as well as co-translational membrane protein insertion. Compared to the individual constituents, the HTL complex is more effective at the insertion of the M13 coat protein Pf3, subunit c of the Fo domain of the ATP synthase, and MscL; all previously thought to be incorporated independently of SecYEG. Interestingly, preliminary data suggests that YidC facilitates membrane protein insertion and assembly, rather than acting as an independent translocon. The structure of the assembly determined by electron cryo-microscopy is equally fascinating. The SecYEG, SecDF-YajC sub-complexes and YidC enclose a large central cavity, which according to small angle neutron scattering (SANS) retains an inner core of lipids. This lipid pool may facilitate the emergence and folding of transmembrane helices prior to release into the bulk of the bilayer. This is an attractive hypothesis because it mirrors the protected folding environment for globular proteins within the water filled interior of GroEL.

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S10.P1

Interactome of LETM1 using miniaturised affinity purification mass spectrometry

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Mitochondrial function is tightly-associated with the morphology and structure of these organelles. One of the many factors that regulate this important feature is ion homeostasis. Several ion channels are responsible for maintaining steady state levels of a number of key ions, namely sodium, potassium and calcium. One protein of particular interest is leucine zipper EF-hand containing transmembrane protein 1 (LETM1). LETM1 is an inner mitochondrial membrane protein that upon knockdown has dramatic effects on mitochondrial function and structure. A role for LETM1 in maintaining potassium homeostasis by regulating a potassium proton exchange has been demonstrated several times [1]. However, based on recent studies, it was suggested that LETM1 may be a calcium proton transporter within the mitochondrial inner membrane [1,2]. To gain a full and robust picture of the involvement of LETM1 in mitochondrial ion homeostasis we decided to investigate proteins that directly interact with LETM1, with the aim of identifying proteins that might regulate or inhibit cation exchange. To do so we used affinity purification (AP) coupled to mass spectrometry. This approach was modified to create a novel method for the identification of interaction partners at an organelle level, namely mitochondrial affinity purifications. By combining this approach with high-end mass spectrometry, we have identified a number of interaction partners of LETM1. Specifically 32 mitochondrial proteins were found to be 2-fold enriched in spectral counts over the GFP control. We are currently validating a number of high-interest targets generated from this approach that illustrate the importance of LETM1 in maintaining not only mitochondrial morphology, but also function.

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S10.P2

Role of *Escherichia coli* hydrogenases in the F0F1-ATPase activity under mixed carbon fermentation at different pHs

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Escherichia coli is able to ferment glycerol producing molecular hydrogen [1]. Various carbon sources fermentation, for instance, mixed glucose and glycerol, could be of interest in biotechnology. *E. coli* is able to synthesize four active [NiFe]-hydrogenases (Hyd 1–3) when grown under anaerobic conditions [1]. Hyd-1 and Hyd-2 are respiratory enzymes catalysing hydrogen oxidation. Hyd-3 and Hyd-4, together with formate dehydrogenase H (Fdh-H), forms the formate hydrogenlyase (FHL) complexes, which are responsible for H2 evolution by intact cells. FHL activity requires H^{+}-FoF1-ATPase, which has been shown to be inhibited by N,N′-dicyclohexylcarbodiimide (DCCD) [2]. In this investigation overall and DCCD-sensitive ATPase activity of membrane vesicles was studied in mixed carbon fermented (glucose and glycerol) *E. coli* wild type and ΔhypF mutant DHP-F2 with deficiency of all Hyd at different pHs. ATPase activity of wild type was ~1.7-fold higher ($p \leq 0.05$) at pH 7.5 compared with that at pH 6.5. It should be noted that ATPase activity of glycerol-fermented wild type was ~11-fold higher at pH 7.5 compared with that at pH 6.5. In mixed carbon grown cells, compared with wild type cells, ATPase activity at pH 7.5 was increased in ~1.3-fold ($p \leq 0.02$) with ΔhypF mutant. DCCD inhibited ATPase activity of ΔhypF mutant markedly ~7-fold ($p \leq 0.05$) at pH 7.5 and ~2-fold at pH 6.5. Note that ATPase activity of wild type and ΔhypF mutant at pH 6,5 was similar. The results indicate that in *E. coli* during mixed carbon fermentation (glucose and glycerol) for the FoF1-ATPase activity alkaline pH is more optimal. F0F1 has major input in overall ATPase activity. The suppression of ATPase activity in ΔhypF mutant at pH 7.5 might be explained by some interaction between FHL complex components with FoF1.

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S10.P3

Loss of NAD^+ through the MUC as an additional mechanism of physiological OxPhos uncoupling in *Debaryomyces hansenii*

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Debaryomyces hansenii is a salt-tolerant yeast that contains two physiological oxidative phosphorylation (OxPhos) uncoupling mechanisms [1]: a) a mitochondrial unspecific channel (MUC) [2] and b) a branched respiratory chain [3]. In exponential phase grown cells, OxPhos are highly coupled and both uncoupling mechanisms inactive. However, upon aging in culture (stationary growth phase), the efficiency of proton pumping decreases and mitochondria become partially uncoupled, probably in an effort to deplete oxygen in the cell without synthesizing ATP. In the stationary phase, the complex I-dependent rate of oxygen consumption and respiratory coupling are selectively decreased. Complex I activity and expression are not changed in this condition. In fact, all other branched respiratory chain components activities remain the same as in the exponential phase. Uncoupled complex I-dependent respiratory activity is due to lack of NAD^+. Here, we propose that NAD^+ possibly escapes the matrix through an open MUC [4]. When NAD^+ is added back, coupled complex I-dependent respiratory activity is recovered in both isolated mitochondria and permeabilized spheroplasts [4]. This uptake seems to be catalyzed by a NAD^+-specific carrier, which is sensitive to bromocresol purple, bathophenanthroline and pyridoxal-5′-phosphate. Loss of matrix NAD^+ through an open MUC is suggested as a novel OxPhos uncoupling mechanism [4].

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S10.P4

Effect of antibody binding on the transport properties of the Na^+/H^+ Antiporter NhaA from *Salmonella Typhimurium*

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Na^+/H^+ antiporters are important for ion homoeostasis of bacteria, which have to survive in rapidly changing or adverse environments. These secondary active transporters, which exchange sodium ions against protons, are crucial for control of intracellular pH, cellular Na^+ concentration and cell volume in all biological kingdoms of life. X-ray structures of NhaA from *Escherichia coli* and of NapA from *Thermus thermophilus* were determined. To obtain further insights in structure/function relationships and in the mechanism of pH-dependent transport, the Na^+/H^+ antiporter NhaA from *Salmonella enterica* serovar Typhimurium LT2 (STNhaA) was crystallized for X-ray structure determination. Following the strategy of antibody fragment mediated crystallization, crystals were obtained with recombinant Fab fragments. Here, we report the characterization of Fab-fragment binding to STNhaA. Purified STNhaA is fully capable of electrogenic Na^+/H^+ antiport when reconstituted in proteoliposomes. Fluorescence-based transport assays with purified, reconstituted STNhaA:Fab complex clearly showed that transport activity is not impaired by binding of the Fab fragment. Mechanistic implications will be discussed.

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S10.P5

Functional and structural characterisation of the human ATP-Mg/Pi carrier

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The ATP-Mg/Pi carrier (APC) is a member of the mitochondrial carrier family of transport proteins, and carries out the counter-exchange of $ATP\text{-}Mg^{2+}$ – for HPO_4^{2-} – between the cytosol and the mitochondrial matrix [1–3]. This is the mechanism for net uptake and efflux of adenosine nucleotides in mitochondria [1–3]. The APC has a trans-membrane domain formed of six helices, characteristic of mitochondrial carrier proteins. The APC also has a unique N-terminal extension, which forms a Ca^{2+} binding regulatory domain [1–3]. The structure of the regulatory domain is known [4], however the mechanism by which calcium regulates transport in the APC is still unresolved. Using biophysical methods such as thermo-stability assays and macromolecular X-ray crystallography the APC has been studied, and new insights about regulation have been gained.

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